Journal of Chromatography, 268 (1983) 528–534
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROM. 16,025

Note

Colour reactions of iodic acid as reagent for identifying drugs by thin-layer chromatography

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(First received May 26th, 1983; revised manuscript received June 1st, 1983)

In previous works[1,2] we studied the action of iodic acid on organic substances, which involves a reaction of either oxidation or substitution according to both the substrate and the experimental conditions. Such an action is favoured by the presence of hydrogen atoms bound to carbon atoms activated by a functional neighboring group[3–6]. In particular, hydrogen atoms on aromatic nuclei with electron-donating groups are very reactive towards iodic acid. The reaction occurs only in strongly acidic media and the rate depends on the concentration of both iodic acid and oxonium ion.

The objective of our work was to examine the reactivity of iodic acid towards many classes of organic compounds of pharmaceutical interest in order to establish if it can be used for detecting and identifying drugs separated by thin-layer chromatography (TLC) on silica gel layers, considering that potassium iodate has been already used successfully for staining sympathomimetic amines[7]. We report here the results of a systematic study of the characteristic chromogenic reactions obtained for various drugs.

EXPERIMENTAL

Materials and methods

Iodic acid (high-purity grade) and the solvents used were supplied by Fluka (Buchs, Switzerland). All the drugs investigated were commercial products and were dissolved in methanol to a concentration of 10 mg/ml.

Chromatographic procedure

An aliquot (10 μl) of each standard solution of substances belonging to the same group was spotted on 0.25-mm pre-coated layers of silica gel G-60 F_{254} (Merck, Darmstadt, F.R.G.). The development was performed at room temperature according to Stahl[8] and Clarke[9]. The developed layers were air-dried, then sprayed with iodic acid reagent. The reagent solution was prepared as follows: 2.0 g of iodic acid were weighed into a 100-ml volumetric flask, dissolved in about 10 ml of water and

0021-9673/83/$03.00

TABLE I

COLOUR REACTIONS OF ANTI-INFLAMMATORIES

– indicates no reaction; + and + + indicate that the colour development is less or more rapid.

Group	*Compound*	*Reaction*
Salicylates	Acetylsalicylic acid	Brown +
Aniline derivatives	Phenacetin	Yellow +
Pyrazolone derivatives	Phenazone	Brown +
	Aminophenazone	Brown +
	Dioxypyramidon	Brown +
	Dipyrone	Yellow +
Anthranilic acids	Floctafenine	Yellow + +
	Mefenamic acid	Yellow + + → dark green
α-Arylpropionic acids	Ibuprofen	–
	Chetoprofen	–
	Fenbufen	Green +
	Indoprofen	–
Arylacetic acids	Alclofenac	Yellow +
	Diclofenac	Violet +
	Furofenac	Black +
Cortisone derivatives	Hydrocortisone acetate	–
	Flunisolide	–
Miscellaneous	Naproxen sodium salt	Brown + +
	Benzydamine	–

then made up to volume with 90% (w/w) sulphuric acid. The iodic acid reagent must be kept in the dark. The colours of the spots were examined immediately after drying of the layer and after 1 and 24 h. The detection limit was determined by spotting 10, 20, 50 and 100 ng of the drugs that gave positive reactions. The plates were not developed but were immediately sprayed with iodic acid. The sensitivity of the chromogenic reaction ranged from 20 to 50 ng for the less reactive compounds.

RESULTS AND DISCUSSION

One hundred and sixty three drugs, classified into eight groups, were examined. The colour reactions after spraying with iodic acid reagent are listed in Tables I–VIII.

From the results it can be concluded that a positive reaction with iodic acid reagent is generally due to the presence in the molecule of an aromatic ring particularly activated towards electrophilic substitution. For example, in the group of anti-inflammatories (Table I) α-arylpropionic acids and cortisone derivatives give no chromatic reaction, in agreement with their structure. The exception of fenbufen is probably due to the presence of a diphenyl group in the molecule. The reactivity of naproxen sodium salt arises, as expected, from the presence of a benzene ring activated by a methoxyl group and fused to another benzene ring.

Among the cardiotonics (Table II), digitalis glucosides are an example of the ability of iodic acid to differentiate molecules having different structures. Proscillaridin, containing an unsaturated δ-lactone ring, gives a characteristic violet colour whereas the other drugs of this group, containing unsaturated γ-lactone rings give a brown colour.

TABLE II

COLOUR REACTIONS OF CARDIOVASCULARS

Symbols as in Table I.

Group	*Compound*	*Reaction*
Cardiotonics	Lanatoside C	Brown + +
	Digitoxin	Brown + +
	Digoxin	Brown + +
	Acetyldigoxin	Brown + +
	β-Methyldigoxin	Brown + +
	Proscillaridin	Violet + +
Cerebral and peripheral vasodilators	Papaveroline	Light brown + +
	Bencyclane	Brown + +
	Papaverine	Orange-yellow + +
	Dimoxyline	Yellow + +
	Vincamine	Olive green + +
	Nafronyl acid oxalate	Brown +
	Butalamine	−
Xanthines	Theophylline	−
Coronary vasodilators	Verapamil	Yellow +
	Amiodarone	Light brown +
	Dipyridamole	−
β-Adrenergic blockers	Alprenolol	Brownish violet +
Antiarrhythmics	Quinidine	−
	Ajmaline	Pink +
	Bunaftine hydrochloride	Yellow
Varices therapy	Ethylbenzyl glycofuranoside	Brown +
Capillary protectives	Tranexamic acid	−
	Aminaftone	Yellow +
Peripheral vasoconstrictors	Synephrine	Brown +
	Octopamine	Brown +
	Etilefrine	Brown +
	Metaraminol	Brown +
	Epinephrine tartrate	Light brown + +
Hypocholesterolaemics	Clofibrate	Rust brown + +
	Pyridinol carbamate	−
	Fenofibrate	Light yellow

Iodic acid reagent is particularly interesting for distinguishing drugs that often are associated in medicinal specialties such as clofibrate and pyridinolcarbamatum (Table III).

Another interesting result was obtained with antibiotics (Table IV). Iodic acid reagent may be advantageously used for the identification, after chromatographic separation, of ampicillin in pharmaceutical associations either with cloxacillins, as the colour reactions are different, or cephalosporins, as the latter show greater reactivity.

Most CNS drugs give a positive reaction with iodic acid (Table V). No positive reaction has been observed between iodic acid and most chemotherapeutics (Table VI). Trimethoprim, PAS and fenticonazolum are exceptions. The positive test obtained with trimethoprim, a pyrimidine derivative, allows its detection in associations with the sulphonamides commonly utilized in medical therapy. As far as indole al-

TABLE III

COLOUR REACTIONS OF GASTROINTESTINAL DRUGS

Symbols as in Table I.

Group	*Compound*	*Reaction*
Antiulcer drugs	Cimetidine	Yellow +
Hepatoprotectives	Citiolone	Dark brown + +
	Cicloxilic acid	Brownish violet + +
	Hymecromone	Light brown + +
	Florantyrone	Light yellow
Hypoglycaemics	Glibencamide	–
	Tolbutamide	–
	Phenformin	–
Antispasmodics	Xenytropium bromide	–
	Fentonium bromide	–
	Homatropine	–
	Mepenzolate	–
	Scopolamine	–
Antidiarrheals	Loperamide hydrochloride	–

TABLE IV

COLOUR REACTIONS OF ANTIBIOTICS

Symbols as in Table I.

Group	*Compound*	*Reaction*
Tetracyclines	Doxycycline	Rust brown + +
	Metocycline	Brown + +
	Minocycline	–
	Demeclocycline	Yellow
	Demethylchlorotetracycline	Yellow + +
	Tetracycline	Brown + +
	Oxytetracycline	Brown + +
Aminoglycosides	Gentamicin	–
	Paromomycin	–
	Aminosidin	–
Penicillins	Ampicillin	Pink-orange +
	Cloxacillin	Dark yellow +
	Dicloxacillin	Yellowish brown +
	Flucoxacillin	Yellowish brown
	Amoxicillin	Light brown +
Cephalosporins	Cephalexin	Yellow-orange + +
	Cefradine	Yellow + +
	Cefacetrile	Mustard yellow + +
	Cephaloridine	Dark brown + +
	Cefazolin	Brown + + → olive green
Macrolides	Rifamycin	Deep pink + → brown
	Erythromycin	Black + +
	Clindamycin	Rust red + +
Polypeptides	Colistin methanesulphonate	–

TABLE V

COLOUR REACTIONS OF CNS DRUGS

Symbols as in Table I.

Group	*Compound*	*Reaction**
Benzodiazepines	Diazepam	Light orange +
	Chlordiazepoxide	Dark brown +
	Nitrazepam	Dark brown +
	Pinazepam	Dark brown +
	Lorazepam	Dark brown +
	Chlordesmethyldiazepam	–
	Flurazepam	–
	Flunitrazepam	–
Barbiturates	Barbital	–
	Phenobarbital	–
	Sodium thiopental	–
Neuroleptics	Trazodone	–
	Thioridazine	Green + +
	Chlorpromazine	Lilac + +
	Promazine	Brick red + +
	Sulpiride	–
	Tiapride	Light yellow
Neuroleptics (non-barbiturates)	Carbamazepine	Yellow-green
	Phenytoin	–
	Beclamide	–
Analgesics—narcotics	Morphine	Yellow +
	Codeine	Yellow + (after 30 min)
	Heroin	Yellow +
	Pentazocine	Yellow
Tricyclic antidepressants	Amitriptyline	Brown + +
	Maprotiline	Brown + +
Anorectics	Amphetamine	Yellow +
	Amfepramone	–
Antiemetics	Metoclopramide	Pink + (after 60 min)
Antihistaminics	Cyproheptadine	Yellowish green +
Local anaesthetics	Cocaine	–
	Amyleine	–
	Procaine	Blue + (after 30 min)
	Lidocaine	–

* Colours obtained after heating at 120°C for 2 min.

kaloids are concerned (Table VII), it can be seen that indole nucleus is very reactive towards iodic acid. In particular, the different behaviour between the natural alkaloids of *Rauwolfia* and semi-synthetic 17-O-acetylajmaline makes it possible rapidly to differentiate the drugs.

It can be concluded that the colour reactions obtained with iodic acid reagent provide additional information to the chromatographic literature which may be useful for tentatively identifying drugs separated by TLC.

TABLE VI

COLOUR REACTIONS OF CHEMOTHERAPEUTICS

Symbols as in Table I.

Group	*Compound*	*Reaction*
Pyrimidine derivatives	Trimethoprim	Violet pink +
Sulphonamides	Sulphapyridine	–
	Sulphamerazine	–
	Sulphadimethoxine	–
	Sulphamethoxazole	–
	Sulphalene	–
Antimalarials	Quinine	–
	Chloroquinine	–
Antituberculotics	PAS	Brown + +
Gout therapeutics	Allopurinol	–
Antifungals	Fenticonazolum	Brown +
Urinary antiseptics	Nalidixic acid	–

TABLE VII

COLOUR REACTIONS OF INDOLE ALKALOIDS

Symbols as in Table I.

Compound	*Reaction*
Reserpine	Green +
Reserpiline	Brown +
Methyl reserpate	Green +
Rescinnamine	Light green +
Yohimbine	Green +
Rauwolscine	Grey-green +
Ajmaline	Pink + → brown (after 12 h)
17-O-Acetylajmaline	Pink + → green (after 12 h)
Dihydroergotoxine	–
Ergotamine	Red +
Nicergoline	Light yellow +

TABLE VIII

COLOUR REACTIONS OF MISCELLANEOUS DRUGS

Symbols as in Table I.

Group	*Compound*	*Reaction*
Myorelaxants	Carisoprodol	–
Mucolytics	Bromhexine hydrochloride	–
	Dropropizine	Orange ++
	Dextromethorphan	Pink +
Vitamins	Nicotinamide	–
	Riboflavine	Yellow ++
	Ascorbic acid	Yellow +
	Thiamine hydrochloride	–
	Pyridoxine hydrochloride	–
	Retinol	Brown ++
	Ergocalciferol	–
Bronchodilators	Salbutamol	Beige +
	Fenspiride	–
Diuretics	Ethacrynic acid	Lemon yellow +
	Spironolactone	Orange ++
	Chlorothiazide	–
	Hydroflumethiazide	–

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